THERAPEUTIC ADVANCES in
Drug Safety

Letter to the Editor

Could chlorophyllins improve the safety profile of beta-d-N4-hydroxycytidine versus N-hydroxycytidine, the active ingredient of the SARS-CoV-2 antiviral molnupiravir?

Ther Adv Drug Saf

2022, Vol. 13: 1–4

DOI: 10.1177/20420986221107753

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Plain Language Summary

Could natural plant pigment (chlorophyll) derivatives (chlorophyllins) improve the safety of the antiviral Molnupiravir, used to treat COVID-19 disease?

Molnupiravir, a specific SARS-CoV-2 antiviral, may cause adverse genetic changes and thereby create potential host cell damage (through genotoxicity and DNA stressors). In our opinion, this side effect of treatment could be reduced if the antiviral was taken as a combined therapy with chlorophyllins. Specifically, we hypothesise that chlorophyllins might improve the overall effectiveness of molnupiravir, typically used to treat patients suffering from COVID-19. Chlorophyllins, antioxidants derived from natural plant chlorophyll, are safe, effective and non-toxic antioxidants that could combat possible genotoxic flow-on effects of molnupiravir. In addition, as they possess antiviral properties, treatment with chlorophyllins may enhance the overall antiviral effect via a mechanism different to molnupiravir.

Molnupiravir (MOV) is an antiviral prodrug used to treat mild to moderate coronavirus 2019 disease (COVID-19) in adults. Targeted to specifically treat those most at risk of severe illness and approved for emergency use by the US Food and Drug Administration in December 2021, MOV is one of many therapeutics used to combat the COVID-19 pandemic.[1] It disrupts the fidelity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genome replication by fostering error accumulation of positive-sense RNA sequences. MOV is the first oral, direct-acting antiviral shown to be highly effective at reducing nasopharyngeal SARS-CoV-2 infectious virus and viral RNA – a powerful and wide-spectrum nucleoside antiviral that is 100 times more potent than either ribavirin or favipiravir.[2,3] The active ingredient of MOV is beta-d-N4-hydroxycytidine (NHC). As a ribonucleoside analogue of cytosine, NHC replaces cytosine and uracil to induce catastrophic coding of viral RNA proteins and thereby prevents viral propagation.[4] Unfortunately, exposure to MOV may induce lethal mutagenesis of mammalian host cell DNA,[5] which has raised significant safety concerns regarding the authorisation of this drug. Specific off-target risks include broad-scale genotoxicity, including modification of the human genome, altered germ cell presentation, selective tumorigenesis, and disease resistance through modification of viral RNA.[2,3,5] The latter even has the potential to prolong the COVID-19 pandemic by generating new SARS-CoV-2 variants that evade host immunity.

Until such time as clinical data are provided to support *in vitro* laboratory findings on which therapeutic administration was approved, concern for toxic mutagenesis induced by MOV and other nucleotide analogues (NAs) will continue to be widely and routinely expressed.[2,5–7] Abrogation of potential risks, especially toxic host cell mutagenicities induced by MOV and other NAs, should include means to reduce oxidative stress.[6] Antioxidant treatments for acute respiratory diseases seldom result in medically

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relevant outcomes, rarely producing repeatable results.[8] When taken alongside antivirals, however, antioxidants are known to intercept NAs[9] without compromising efficacy, and, in many cases, even having an enhancing effect.[10] When taken in combination with antivirals,[11,12] oxidative stress is reduced. Thus, combined therapy of antioxidants and MOV could generate antimutagenic effects, thereby improving the safety profile of NAs such as NHC. Clinical advancement of combined drug therapy of antioxidants and NAs largely focuses on chronic viral infections such as hepatitis C.[10,11] However, small-scale *in vivo* studies do indicate co-administration of antioxidants and NAs improve treatment outcomes in mice with respiratory distress induced by lethal influenza.[12]

Known mostly as a cancer treatment, chlorophyllins are powerful antioxidants that confer antiviral properties.[13,14] As therapeutics, chlorophyllins provide a direct dose-dependent therapeutic effect, largely attributable to broad-scale antioxidant activity.[14] Derived from natural chlorophyll α, the central magnesium ion is most often replaced with copper, iron or zinc.[15] Capable of enhancing proliferative properties of immune-modulating proteins, chlorophyllin therapy can prevent many diseases or improve treatment outcomes, including for immune proliferative disorders, hyperinflammation, pathogenic viruses, fungi and bacteria.[16] Importantly, unlike several dietary antioxidants, and excluding potential embryotoxicity,[17] high doses of chlorophyllins, notably sodium copper chlorophyllin (SCC) and sodium zinc chlorophyllin (SZC), are not toxic when administered by a variety of routes. In fact, metal toxicity induced by chlorophyllins has never been reported, even when such doses greatly surpass recommended intake of the central ion.[18] Significantly, patient outcomes of treating with chlorophyllins are frequently reported as beneficial or show greater efficacy than other medications typically prescribed for the same purpose, for example, equivalent treatment for leukopenia.[19,20] Some therapeutics, such as NHC, may cause imbalance to steady-state intercellular redox reactions[5,6] and toxic mutagenesis.[7] In contrast, several derivatives of chlorophyll α effectively mediate cell mutagenicities and cytotoxicities caused by harmful reactive oxygen species (ROS), including reactions that preserve mitochondrial function.[21–23] Notably, chlorophyllins can prevent exogenous damage caused by plant toxins and ultraviolet radiation.[14] Likewise, chlorophyllins may abrogate cytotoxicities that lead to mutagenesis, as they act to prevent endogenous nuclear mutagenesis induced by ROS.[24,25]

Although mechanisms by which chlorophyllins control steady-state redox reactions in the host cell environment remain unclear[13] several studies suggest that chlorophyllins alone as well as with a centralised metal ion, such as copper and zinc, contribute critically to this process.[22] Essential to immune competence and pathogenic control, both metals play a host modular role in mediating inflammatory and apoptotic events, binding to several enzyme complexes that produce ROS.[22] As key contributors to antioxidant states, copper and zinc could be enhanced by chlorophyllin and other chlorophyll α compounds,[15,22] thereby enhancing natural defence systems and intercepting inadvertent host cell mutagenesis. Despite producing a greater antioxidant effect, chorophyllins are rarely recognised alongside dietary antioxidant compounds such as vitamin A, C and E. For instance, chlorophyllins containing both copper and zinc produce enhanced antioxidant activity when compared with vitamin C.[20,21] Moreover, unlike dietary antioxidants, this protective effect extends to significant dose-dependent therapeutic outcomes, such as when treating leukopenia,[20] cancer[26] and viral infections.[16] Furthermore, chlorophyllins protect mitochondrial DNA in human cells, including lymphocytes indicating strong protective effects against destruction of lymphocytes and mutagenesis of epithelial lung cell tissue.[24,25]

Chlorophyllins could be used to improve the safety profile of NAs such as those used to treat SARS-CoV-2, including – but not limited to – MOV. Chlorophyllins taken orally, during or post treatment, could mitigate the genotoxic effect of NHC produced by MOV and other NAs, providing a much sought-after protective antioxidant effect that could restore function to excision of DNA repair enzymes.[11] In turn, this may initially deliver a more targeted antiviral approach that does not generate toxic by-products in the host cell. As other derivatives of chlorophyll α, such as pheophorbide α, are already recognised for their antiviral effect against SARS-CoV-2,[27,28] the varied protection afforded by these compounds suggests that chlorophyllins are likely to safely enhance the therapeutic efficacy of NAs. Unlike

antioxidants, which do not possess antiviral properties, the broad-spectrum antiviral effects of chlorophyllins could act as an adjuvant, whereby delivery in combination with MOV may provide an enhanced antiviral effect via two entirely distinct mechanisms.[16,29] Moreover, combined treatment may also enhance natural defence systems, abrogating oxidative damage and inflammatory effects that are caused not only by NHC but also SARS-CoV-2.[30]

In light of what we have discussed, it is our informed opinion that oral co-administration of chlorophyllins such as SCC and SZC should be considered as a way to improve the overall safety and tolerability profile of the SARS-CoV-2 antiviral MOV. The presence of chlorophyllins may abrogate toxic side effects such as host cell mutagenesis produced by NHC, the active ingredient of MOV. Combined therapy in patients may provide the additional benefit of reducing harmful ROS produced by NHC and as a response to viral infection. The outcome would be to restore immune homeostasis and to trigger DNA repair by enhancing the efficacy of MOV and potentially providing a combined antiviral effect delivered by both chlorophyllins and MOV.

Declarations

Ethics approval and consent to participate

Our study did not require ethics board approval because it is an opinion piece based on rigorous observation of existing literature.

Consent for publication

Not applicable.

Author contributions

Nicole F. Clark: Conceptualization; Formal analysis; Investigation; Writing – original draft; Writing – review & editing.

Andrew W. Taylor-Robinson: Project administration; Supervision; Validation; Writing – review & editing.

Kirsten Heimann: Project administration; Supervision; Validation; Writing – review & editing.

Acknowledgements

None.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Competing Interests

The authors declare that there is no conflict of interest.

Availability of data and materials

Not applicable.

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